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Recovery from postischemic acute renal failure in the rat

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Recovery from postischemic acute renal failure in the rat. To define the pattern of recovery from postischemic acute renal failure (ARF), we performed clearance and micropuncture studies at intervals of 1, 2, 4, and 8 weeks following 60 min of complete unilateral renal artery occlusion in the rat. At 1 week, the inulin clearance (C_{In}) of the postischemic kidney was less than 2% of normal. The presence of marked preglomerular vasoconstriction was indicated by the reductions in renal blood flow (RBF), and stop-flow (SFP) and estimated glomerular capillary hydrostatic pressures (GCP_e). In addition, there was evidence of tubular obstruction. Proximal intratubular pressures (PITP) were elevated, and intratubular casts could be seen in vivo and on histologic sections. At 2 weeks, C_{In} had increased more than tenfold. This change occurred in the absence of any significant elevation in RBF, SFP, or GCP_e. PITP had fallen, however, to normal values, and histologic sections revealed a marked reduction in the extent of intratubular casts. Ipsilateral urinary recovery of ^{3}H-inulin microinjected into proximal convolutions was complete. At 4 and 8 weeks, there were further but more gradual rises in C_{In}, which were associated with progressive increases in RBF, SFP, and GCP_e. These observations indicate that recovery from postischemic ARF occurred in a biphasic pattern. The initial rise in C_{In} was associated with the relief of intratubular obstruction, whereas subsequent rises in C_{In} occurred in association with progressive renal vasodilatation.

Evolution de l'insuffisance rénale aiguë postischémique chez le rat. Afin de définir les modalités de l'évolution de l'insuffisance rénale aiguë (ARF) postischémique, des clearances et des microponctions ont été réalisées à des temps 1, 2, 4, et 8 semaines après l'occlusion complète d'une artère rénale pendant 60 min chez le rat. A une semaine la clearance de l'inuline (C_{In}) du rein postischémique est inférieure à 2% de la valeur normale. L'existence d'une vasoconstriction préglomérulaire importante est indiquée par les diminutions du débit sanguin rénal (RBF), de la pression de stop-flow (SFP) et de la pression hydrostatique capillaire (GCP_e) calculée. De plus, des signes d'obstruction tubulaire sont observés. Les pressions intratubulaires proximales (PITP) sont augmentées et des cylindres intratubulaires peuvent être observés in vivo et sur les coupes histologiques. A deux semaines, C_{In} a plus que décuplé. Cette modification survient en l'absence de toute augmentation significative de RBF, SFP, ou GCP_e. PITP, cependant, est revenue aux valeurs normales et les coupes histologiques montrent une diminution considérable des cylindres intra-tubulaires. La récupération ipsilatérale de l'inuline tritiée injectée dans les convolutions proximales est complète. A 4 et 8 semaines, il y a une augmentation supplémentaire mais plus lente de C_{In} qui est associée à des augmentations progressives de RBF, SFP, et GCP_e. Ces constatations indiquent que l'évolution de l'ARF postischémique est biphasique. L'augmentation initiale de C_{In} est contemporaine de la levée des obstructions intra-tubulaires alors que l'augmentation ultérieure survient en même temps qu'une vasodilatation progressive.

A number of recent advances have been made in the understanding of the pathophysiologic mechanisms responsible for the initiation and maintenance of acute renal failure. These studies, which have been reviewed in detail [1, 2], have indicated clearly that a variety of complex and possibly interrelated abnormalities play important roles in the decrease in renal function. It also has become apparent that these abnormalities may change with time and are to some extent dependent upon the method by which the renal damage is produced.

This situation is illustrated by the sequence of events following the induction of postischemic acute renal failure in the rat by 60 min of unilateral complete renal artery occlusion [3–5]. This maneuver results in severe functional alterations marked by an early phase of tubular obstruction that is followed by intense preglomerular vasoconstriction. Renal function is further compromised by changes in the permeability characteristics of the tubular epithelium, which permit the passive backflow of filtrate [3, 4, 6–8].

Despite these observations, little is known concerning the events leading to recovery or the extent to which recovery occurs. Because the presence of the contralateral kidney permits sequential studies of the function of the postischemic kidney, we examined renal function at 1, 2, 4, and 8 weeks following unilateral ischemia. Our results indicate that recovery occurs in a biphasic pattern, with an early phase most closely associated with the return of anatomical and functional integrity of the tubular epithelium and the relief of intratubular obstruction, and a later phase marked by decreases in the degree of preglomerular vasoconstriction.

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Methods

Observations are reported on a total of 37, male, Sprague-Dawley rats studied at intervals of either 1, 2, 4, or 8 weeks after unilateral acute renal failure was produced by clamping the left renal artery for 1 hour. Prior to clamping, unfasted animals had been anesthetized with sodium pentobarbital, 60 mg/kg of body weight, placed on a heated table that maintained body temperature between 37° C and 38° C, and their left kidneys exposed through a small subcostal incision. The left renal artery was isolated, 10 to 15 U/100 g body weight of heparin (1,000 U/ml, The Upjohn Co., Inc.) was injected intravenously, and a small, smooth surfaced tension clamp (Schwartz 1-inch clip, Roboz Surgical Instruments, Co., Inc.) was positioned on the artery so that it was completely occluded. The clamp was removed after 1 hour, the incision closed, and the rats returned to their cages. Saline (0.85% sodium chloride) was infused intravenously at 40 μl/min for 1 hour before and after occlusion; the rate of infusion during the ischemic period was reduced to 20 μl/min.

Following recovery from anesthesia, the animals were maintained on their standard rat pellet diet (Purina) until the evening prior to study when, along with an additional group of 10 normal animals, they were allowed access to water only. The rats were anesthetized by intraperitoneal injection of sodium pentobarbital, 60 mg/kg of body weight, and placed on a heated table. A tracheostomy was performed, and catheters were placed into a femoral vein for the infusion of 0.85% sodium chloride solution at a rate of 40 μl/min. The left kidney was exposed through an abdominal incision for micropuncture as previously described [9]. The renal capsule, the covering peritoneum, and the perirenal fat were left undisturbed. Both ureters were catherized with PE-10 polyethylene tubing. Femoral arterial blood pressure (ABP) was recorded continuously with a Statham P23Db pressure transducer connected to a Beckman recorder.

After the surgical procedures were completed, appropriate amounts of inulin and paraaminohippurate (PAH) were added to the saline infusion. An equilibration period of 1 hour was allowed to elapse, and initial measurements were performed during two 30-min periods. The ureteral catheters were then replaced by PE-50 polyethylene tubing, and the rats were acutely expanded with isotonic saline at 1 ml/min for 5 min and then maintained at 400 μl/min for the duration of the experiment. Post-expansion observations commenced 30 min after administration of the priming solution and continued for two additional 30-min periods.

Because previous studies [10] had indicated that clearance methods may not be reliable determinants of renal blood flow (RBF) following temporary ischemia, we compared the values of RBF, as determined by the PAH clearance technique [$RBF_{PAH} = C_{PAH}/E_{PAH}\,(1 - Hct)$], with those simultaneously determined using a small-diameter flow transducer (EP model 401.5; lumen size, 1.5 mm in circumference) connected to a square-wave electromagnetic flowmeter (model 501, Carolina Medical Electronics, Inc.) and a Beckman dynograph (RBF_{direct}). The flow meter system was calibrated in vitro periodically throughout the study as previously described [11]. The extraction ratio of PAH [$E_{PAH} = (A_{PAH} - V_{PAH})/A_{PAH}$] at 1 week was not significantly different from zero at 6 ± 3% ($N = 5$), and as a result, no correlation existed between RBF_{PAH}/RBF_{direct}. Consequently, RBF_{direct} is reported for those animals studied at 1 week. In contrast, E_{PAH} was 60 ± 5% at 2 weeks ($N = 10$), 62 ± 4% at 4 weeks ($N = 10$), and 70 ± 6% at 8 weeks ($N = 7$). Although these values are significantly less than E_{PAH} of normal kidneys (84 ± 2%; $N = 9$), the ratio of RBF_{PAH}/RBF_{direct} determined in a total of 10 animals studied at 2, 4, and 8 weeks was not different from unity, with a best-fit regression line of $RBF_{PAH} = 0.08 + 0.98\,RBF_{direct}$ ($r = 0.762$; $P < 0.01$). These data indicate that the PAH clearance technique is a reliable determinant of RBF by week 2 following temporary ischemia. Since use of the flow transducer may be associated with changes in urine flow rates [11], RBF_{direct} was not determined in all animals. Consequently, RBF_{PAH} is reported for those animals studied at 2, 4, and 8 weeks.

Hydrostatic pressure in random surface proximal convolutions was measured using sharpened glass pipettes (3 to 7 μ O.D.) filled with 2 M sodium chloride and a continuously recording, electronic servonulling apparatus. The stop-flow hydrostatic pressure (SFP) was measured in the earliest accessible loop of a proximal tubule as previously described [12, 13]. From these pressures, afferent effective filtration pressure was estimated as $AEFP_e = SFP - PITP$, where PITP is proximal intratubular pressure. Glomerular capillary pressure (GCP_e) was estimated from the sum of the SFP and the afferent colloidal osmotic pressure (πa), which is assumed to equal systemic plasma oncotic pressure.

During the period of volume expansion, microinjections into random surface proximal convolutions were performed in animals studied 1 and 2 weeks following ischemia, and the results were compared to those performed in normal kidneys of rats undergoing a similar period of expansion. Con-

striction micropipettes with a tip diameter of 4 to 6 μ and of known volume were used. A small volume (1.0 to 4.5 nl) of isotonic saline stained with nigrosin and containing either ^{3}H-inulin (ICN Pharmaceuticals Corporation, Inc.) or ^{3}H-inulin and ^{14}C-mannitol (New England Nuclear) was microinjected at low rates (2 to 10 nl/min). An injection was considered satisfactory if the injection rate could be controlled to avoid visible retrograde flow of the dye-stained test solution and dilatation of the tubular lumen.

Ureteral urine from both kidneys was collected into vials containing 10 ml of PCS solubilizer (Amersham/Searle Corporation) and 1 ml of water. Urine collections were started at the beginning of each microinjection, and four consecutive 5-min collections were made after injections. Before each microinjection, urine was collected to measure residual radioactivity. Immediately preceding the microinjection, timed urine collections of the same duration as each sample after microinjection were taken. A volume of test solution equal to that which was subsequently injected was deposited into these samples and used as reference standards. Radioactivity was measured for 20 min in a 3-channel liquid scintillation spectrometer.

Blood samples were obtained at the midpoint of each period from the tip of the tail, and hematocrit was measured in heparinized capillary tubes. Plasma protein concentration was determined with rat plasma total protein standards by an adaptation of the Lowry technique [14], and colloidal osmotic pressure was calculated with the Landis-Pappenheimer equation [15]. Serum and urinary solution concentrations were determined using a Zeiss PMQ II flame-emission spectrophotometer (Carl Zeiss, New York). Plasma and urine inulin concentrations were determined by the anthrone method [16], whereas plasma and urine PAH concentrations were determined by the Battron and Marshall techniques [17] as modified by Smith et al [18].

At the end of the experiment, both kidneys were excised, cleared of perirenal fat, decapsulated, and weighed immediately. Several kidneys from each observation period were fixed in Helly's solution for histologic examination. Results are expressed as means ± SEM. For analysis of significance, we used the paired and unpaired Student's t tests and linear regression by the least square method. A P value greater than 0.05 was considered not to be statistically significant (NS).

Results

At the time of the renal artery occlusion, there was no significant difference in the body weights of those animals subsequently studied at 1, 2, 4, and 8 weeks (Table 1). Although a tendency existed for the animals to lose weight during the first week after temporary renal artery occlusion, this trend was less apparent during the week 2, and by the weeks 4 and 8, mean body weight had increased. In contrast, the left kidney, which at week 1 was significantly heavier than either the contralateral right kidney or the left kidney of control animals,

Table 1. Variations in body weight, kidney weight, hematocrit, and plasma protein concentration during recovery from postischemic acute renal failure[a]

	Weeks after occlusion				
	1	2	4	8	Control
Body weight, *g*					
At occlusion	249 ± 20	274 ± 18	246 ± 5	261 ± 12	—
At study	235 ± 16	264 ± 14	270 ± 10	311 ± 8[b, c, d]	272 ± 4[b, e]
Kidney weight, *g*					
Left	1.68 ± 0.06	1.27 ± 0.10[b]	0.87 ± 0.06[b, c]	0.74 ± 0.10[b, c]	1.02 ± 0.04[b, c, e]
Right	1.26 ± 0.06	1.24 ± 0.08	1.12 ± 0.05	1.18 ± 0.06	0.96 ± 0.02[b, c, d, e]
Hematocrit, *ml/100 ml*					
During hydropenia	48 ± 1	50 ± 1	52 ± 1[b]	53 ± 1[b]	54 ± 1[b, c]
During volume expansion	42 ± 2	41 ± 2	44 ± 1	—	—
Plasma protein concentration, *g/100 ml*					
During hydropenia	4.58 ± 0.19	5.18 ± 0.14[b]	5.32 ± 0.21[b]	5.80 ± 0.36[b]	4.96 ± 0.15[e]
During volume expansion	2.94 ± 0.06	3.40 ± 0.06[b]	3.47 ± 0.11[b]	—	—
No. of animals					
During hydropenia	10	10	10	7	10
During volume expansion	7	7	7	—	—

[a] Results are means ± SEM.
[b] $P < 0.05$ vs. 1 week.
[c] $P < 0.05$ vs. 2 weeks.
[d] $P < 0.05$ vs. 4 weeks.
[e] $P < 0.05$ vs. 8 weeks.

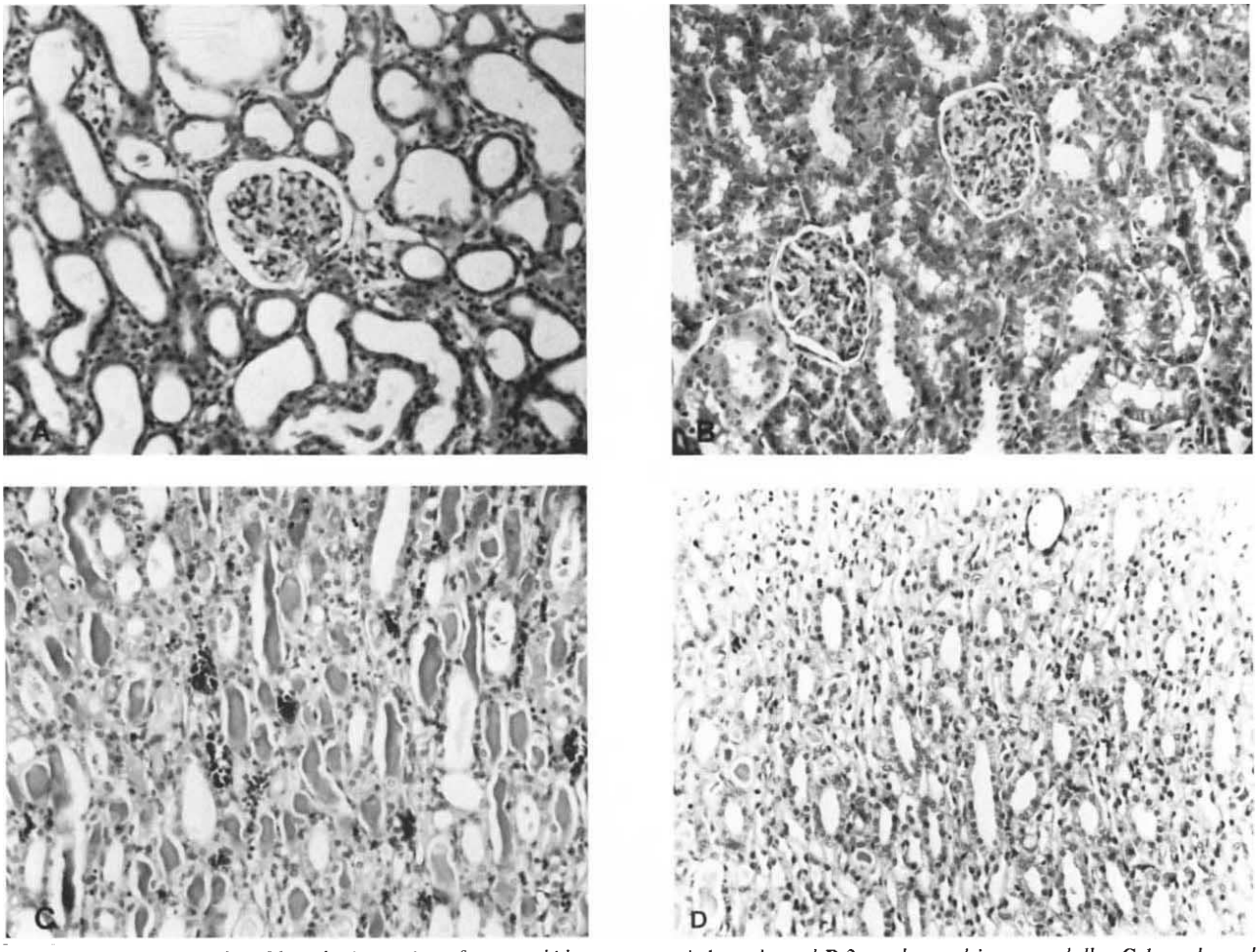

Fig. 1. *Photomicrographs of histologic sections from rat kidney cortex,* **A** *1 week, and* **B** *2 weeks, and inner medulla,* **C** *1 week, and* **D** *2 weeks, following 1 hour of complete renal artery occlusion (hematoxylin and eosin stain; magnification, ×66).*

progressively lost weight with time, so that by week 8 it was significantly less than was the weight of either kidney. Both the hematocrit and plasma protein concentrations rose progressively throughout the period of observation. The degree of volume expansion, as reflected by changes in the hematocrit, and plasma protein concentrations were similar at each interval.

One week after ischemia. One week following occlusion, the in vivo appearance of the postischemic kidney was similar to that previously described [19]. The kidney appeared enlarged with a smooth capsule. Blood could be seen flowing through the peritubular vessels at a diminished rate. Although a small percentage of the tubular lumens were narrow, most were dilated with white amorphous material scattered throughout the surface convolutions. Intravenously injected FD & C green dye appeared faintly in proximal tubules and moved downstream in an extremely sluggish fashion, as did intratubularly injected oil droplets. Examination of histologic sections revealed normal appearing glomeruli (Fig. 1a). The tubules were dilated with flattened tubular epithelium and were separated one from another by a prominent round cell infiltrate in the interstitium. A most striking abnormality was the presence of intratubular casts, which were scattered throughout the cortex and were present in large numbers in the renal papilla (Fig. 1c).

Clearance data are listed in Table 2. The inulin clearance (C_{In}) of the postischemic kidney was less than 2% that of the contralateral right kidney and the left kidney of control animals. Although volume expansion resulted in a 125% increase in C_{In}, the absolute change was small at 19 ± 2 μl/min. Renal vascular resistance (RVR) was markedly elevated, as indicated by the reduction in RBF to 26% of control values. Although RBF increased by more than 40% with volume expansion, it did not return to normal. This response contrasts to that noted 22 to 25 hours following ischemia [4] when volume expansion resulted in the normalization of both RVR

Table 2. Clearance determinations during recovery from postischemic acute renal failure[a]

	Weeks after occlusion				
	1	2	4	8	Control
Left (postischemic) kidney					
Inulin clearance, *μl/min*					
During hydropenia	15 ± 4	178 ± 34[b]	234 ± 40[b]	429 ± 95[b]	817 ± 54[b, c, d, e]
During volume expansion	34 ± 6	278 ± 44[b]	346 ± 47[b]	—	—
Renal blood flow, *ml/min*					
During hydropenia	1.4 ± 0.3	1.7 ± 0.2	2.1 ± 0.2	3.5 ± 0.8[b]	5.4 ± 0.3[b, c, d, e]
During volume expansion	2.0 ± 0.3	2.7 ± 0.5	2.8 ± 0.3	—	—
Urine flow, *μl/min*					
During hydropenia	1.8 ± 0.5	4.2 ± 1.3	1.3 ± 0.3[c]	2.2 ± 0.7	3.5 ± 0.3[b, d]
During volume expansion	10.9 ± 2.2	32.4 ± 5.2	7.4 ± 0.4	—	—
Urine sodium concentration, *mEq/liter*					
During hydropenia	141 ± 21	88 ± 14	44 ± 7[c]	46 ± 13[b]	26 ± 4[b, c, d]
During volume expansion	132 ± 17	188 ± 40	205 ± 38	—	—
No. of animals					
During hydropenia	10	10	10	7	10
During volume expansion	7	7	8	—	—
Right (nonischemic) kidney					
Inulin clearance, *μl/min*					
During hydropenia	1182 ± 182	1148 ± 100	1078 ± 78	1356 ± 136	907 ± 42[c, e]
During volume expansion	1236 ± 118	1249 ± 149	1310 ± 104	—	—
Urine flow, *μl/min*					
During hydropenia	4.2 ± 0.7	5.4 ± 1.4	3.8 ± 0.6	5.2 ± 1.3	4.1 ± 0.4
During volume expansion	33.0 ± 8.6	37.6 ± 6.1	20.4 ± 3.2[c]	—	—
Urine sodium concentration, *mEq/liter*					
During hydropenia	25 ± 16	26 ± 4	35 ± 5	40 ± 15	34 ± 26
During volume expansion	170 ± 29	158 ± 28	232 ± 30	—	—
No. of animals					
During hydropenia	9	10	10	7	10
During volume expansion	7	7	8	—	—

[a] Results are means ± SEM.
[b] $P < 0.05$ vs. 1 week.
[c] $P < 0.05$ vs. 2 weeks.
[d] $P < 0.05$ vs. 4 weeks.
[e] $P < 0.05$ vs. 8 weeks.

and RBF. During hydropenia, urine volume (V) was significantly less from the left kidney than it was from the right ($P < 0.05$). The urinary sodium concentration (U_{Na}) was much greater on the left than it was on the right ($P < 0.001$). Consequently, the fractional excretion of sodium (FE_{Na}) (Fig. 2) was markedly elevated on the left at 10.09 ± 3.58%, as compared to 0.08 ± 0.03% on the right ($P < 0.005$). FE_{Na} of control animals was 0.08 ± 0.02 and 0.11 ± 0.04 in the left and right kidneys, respectively.

Table 3 summarizes the determinations of hydrostatic pressure. PITP were elevated and were more heterogenous than were those of normal (Fig. 3). The SFP and GCP_e were significantly lower than were control values. As a result, the $AEFP_e$ was markedly depressed. Volume expansion did not produce a significant change in any of these values. Microinjections were attempted in several animals; because of the presence of intratubular obstruction, as indicated by the high intratubular pressure and the sluggish tubular fluid flow, they were not technically satisfactory, and consequently, the results are not reported.

Two weeks after ischemia. Two weeks following occlusion, there was a somewhat granular appearance to the kidney surface, although capillary blood flow appeared brisk. The surface tubules could be divided into three distinct populations on the basis of their in vivo microscopic appearance. Approximately three fourths of the tubules were relatively normal in appearance, although the epithelial cells were prominent. Tubular fluid flow was brisk. The remaining tubules were either grossly dilated and tortuous with sluggish tubular fluid flow or were filled with white amorphous material without evidence of tubular flow. Histologic sections (Fig. 1, b and d) also showed considerable variability in tubular size. Although the majority of tubules were no longer distended, a few remained widely patent. Intratubular casts could be seen in the cortex, medulla, and renal papilla. They were markedly decreased in numbers, however, when compared to those seen 1 week after ischemia.

These anatomical changes were associated with functional improvement as indicated in Table 2. When compared to the animals studied 1 week after

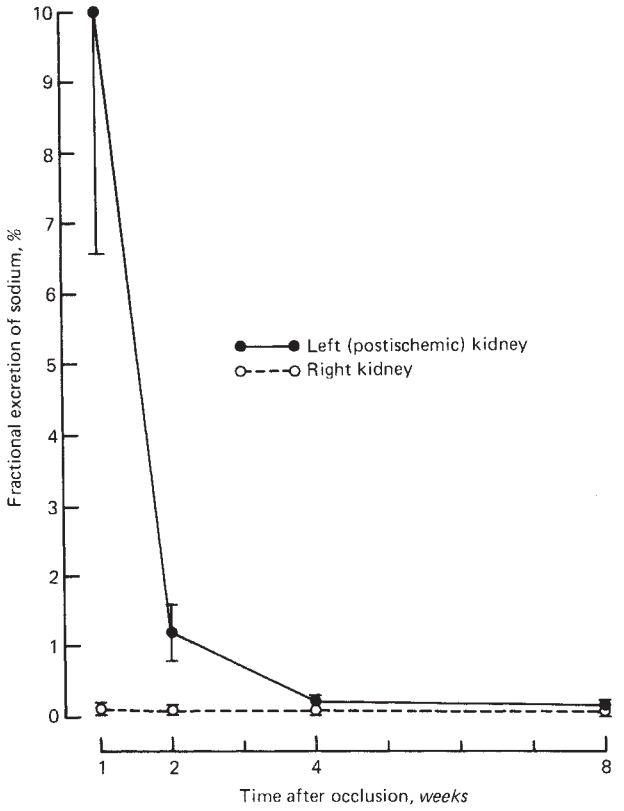

Fig. 2. *Progressive decrease in fractional excretion of sodium from postischemic kidney 1, 2, 4, and 8 weeks after 1 hour of complete unilateral renal artery occlusion.*

occlusion, the C_{In} of the postischemic left kidney had increased more than tenfold to a value of 15% of the nonischemic right kidney (22% of control). This rise in C_{In} occurred out of proportion to the change in RBF (Fig. 4). Whereas the mean value for RBF was slightly higher than was that observed at 1 week, the difference was not statistically significant. In response to volume expansion, the increase in C_{In} of 114 ± 28 μl/min was significantly greater than was that seen at 1 week ($P < 0.005$). The change in RBF was 1.16 ± 0.40 ml/min and was not different from that observed at 1 week. In these animals, the urine volume of the left kidney tended to be greater than it was at any other time. This difference was most impressive following volume expansion, when the urine flow rate was nearly three times that seen under similar circumstances at 1 week. The U_{Na} tended to be lower than was that observed earlier, but it remained greater than was that of the right kidney ($P < 0.001$). Likewise, the FE_{Na} at 1.2 ± 0.4% (Fig. 2) was lower than it was at 1 week ($P < 0.025$) but greater than was the FE_{Na} of the right lower kidney ($P < 0.02$) which remained unchanged at 0.08 ± 0.02%.

The increase in C_{In} occurred in the absence of changes in either the SFP or GCP_e (Table 3). There was, however, a significant fall in the mean PITP, which was apparent during both hydropenia and

Table 3. Hydrostatic pressures during recovery from postischemic acute renal failure[a]

	Weeks after occlusion				
	1	2	4	8	Control
Proximal intratubular pressure, *mm Hg*					
During hydropenia	17.5 ± 2.2	10.4 ± 0.9[b]	11.9 ± 0.4[b]	11.0 ± 0.4[b]	11.8 ± 0.8[b]
During volume expansion	19.8 ± 2.5	12.9 ± 1.3[b]	13.4 ± 2.0	—	—
Stop-flow pressure, *mm Hg*					
During hydropenia	24.0 ± 2.0	24.2 ± 1.4	26.4 ± 1.1	30.8 ± 1.9[b, c, d]	34.8 ± 2.0[b, c, d]
During volume expansion	26.5 ± 2.5	28.1 ± 1.2	33.1 ± 2.2	—	—
Afferent effective filtration pressure, *mm Hg*					
During hydropenia	6.6 ± 1.2	13.8 ± 0.8[b]	14.5 ± 0.9[b]	19.8 ± 1.7[b, c, d]	23.0 ± 1.5[b, c, d]
During volume expansion	6.7 ± 0.9	15.2 ± 2.1[b]	19.7 ± 1.6[b]	—	—
Estimated glomerular capillary pressure, *mm Hg*					
During hydropenia	38.0 ± 2.0	40.7 ± 1.4	43.6 ± 1.0[b]	50.4 ± 3.4[b, c, d]	50.4 ± 2.3[b, c, d]
During volume expansion	34.3 ± 2.7	37.4 ± 1.2	42.7 ± 2.1[b, c]	—	—
Femoral arterial blood pressure, *mm Hg*					
During hydropenia	113 ± 6	117 ± 6	116 ± 4	108 ± 6	117 ± 3
During volume expansion	98 ± 6	108 ± 5	110 ± 4	—	—
No. of animals					
During hydropenia	10	10	10	7	10
During volume expansion	7	7	7	—	—

[a] Results are means ± SEM.
[b] $P < 0.05$ vs. 1 week.
[c] $P < 0.05$ vs. 2 weeks.
[d] $P < 0.05$ vs. 4 weeks.
[e] $P < 0.05$ vs. 8 weeks.

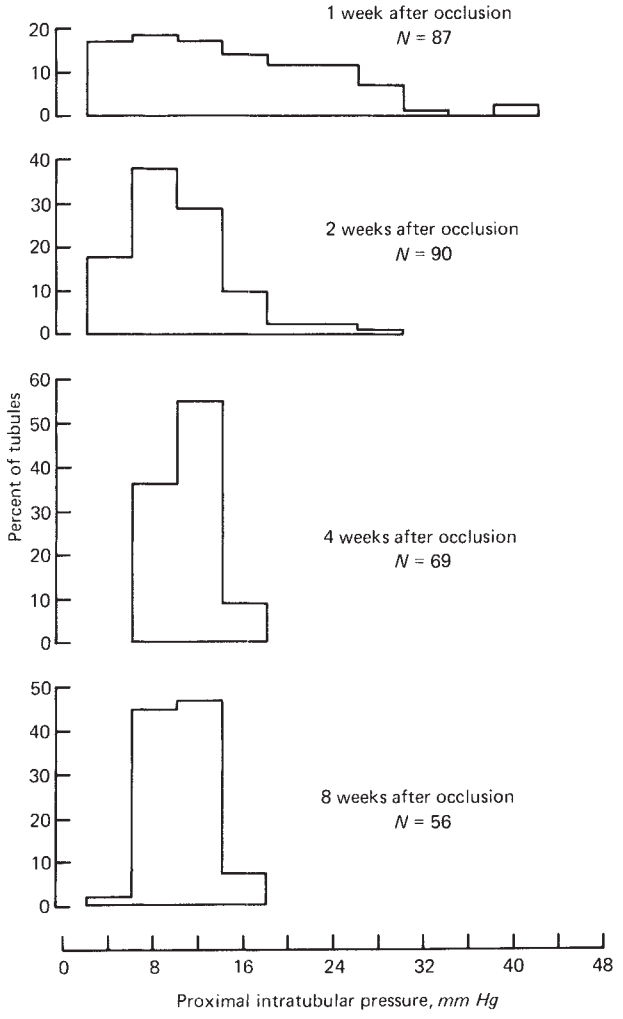

Fig. 3. *Frequency distributions of proximal intratubular pressures measured 1, 2, 4, and 8 weeks after 1 hour of complete unilateral renal artery occlusion.*

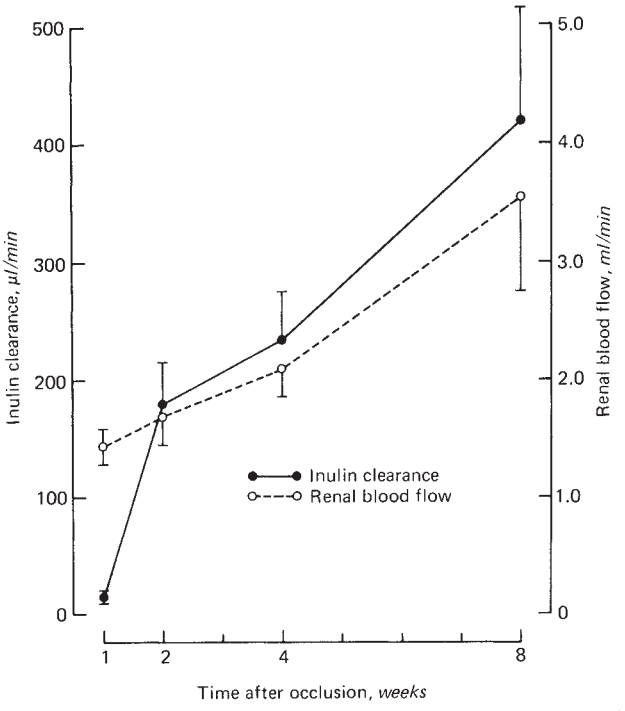

Fig. 4. *Progressive increase in inulin clearance and renal blood flow of postischemic kidney 1, 2, 4 and 8 weeks after 1 hour of complete renal artery occlusion.*

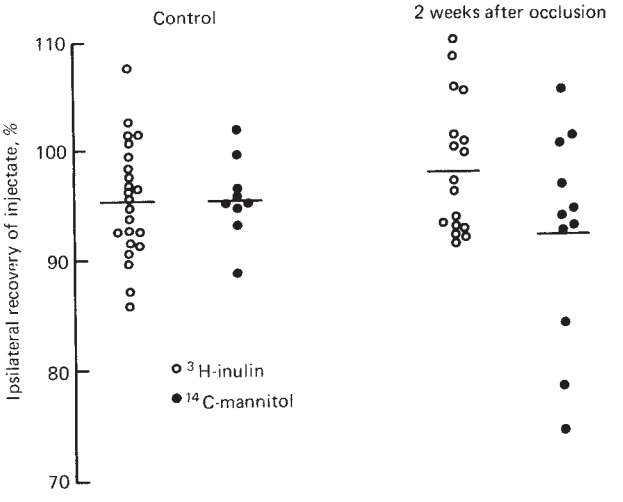

Fig. 5. *Fractional inulin and mannitol recoveries from postischemic kidney after simultaneous proximal microinjection 2 weeks after 1 hour of complete unilateral renal artery occlusion.*

volume expansion. Although the individual values continued to be more heterogenous than normal, they were less heterogenous than were those measured earlier (Fig. 3). Consequently, the increase in the mean $AEFP_e$ was entirely due to the fall in PITP.

A total of 17 technically satisfactory microinjections were performed in 9 animals and the results compared to a similar group of microinjections performed in normal kidneys. The results presented are recoveries in the urine from the injected kidney. The recovery of inulin after proximal injection is shown in Fig. 5. Two weeks after occlusion, recovery averaged 97.8 ± 1.4% (N = 17). In control animals, recovery was similar, averaging 95.4 ± 1.1% (N = 23). Neither value is significantly different from 100%. The recovery of mannitol simultaneously injected with inulin into proximal convolutions is also shown in Fig. 5. In the control animals, recovery of mannitol averaged 94.5 ± 1.6% (N = 9) and was not significantly different from the inulin recovery. Two weeks after occlusion, however, the recovery of mannitol averaged 91.8 ± 2.9% (N = 11) and was significantly less than was that of inulin (P

< 0.05). In several instances, mannitol recovery was less than 85%.

Four and eight weeks after ischemia. In those animals studied 4 and 8 weeks following renal artery occlusion, the kidney surface was slightly irregular. Whereas capillary blood flow was brisk, the vessels were not as prominent as normally seen. Occasional tubules were dilated or contained intratubular casts, although they were less apparent than were those seen earlier. A similar pattern was seen in histologic sections.

In contrast to the rapid rise in C_{In} that occurred between the first and second week following ischemia, the rise in C_{In} that occurred between the second and eighth weeks was more gradual and tended to parallel the rise in RBF (Fig. 4). By the fourth week, the C_{In} of the left kidney was 22% that of the right kidney (29% of control), and by the eighth week it was 31% that of the right kidney (52% of control). At both 4 and 8 weeks, U_{Na} (Table 2) and the FE_{Na} (Fig. 2) reached levels similar to those seen in the nonischemic right kidney. At each interval, urine flow rates from the left kidney were less than were those from the right kidney.

There was no significant difference in the mean PITP noted at 4 or 8 weeks following ischemia (Table 3), although the individual values were less heterogenous than those measured earlier (Fig. 3). There was, however, a progressive rise in the SFP and GCP_e, so that at 8 weeks the values were significantly greater than were those observed at other intervals, and not statistically different from control values. This rise in SFP and GCP_e accounted for the progressive increase in the $AEFP_e$ observed between the second and eighth week following ischemia.

Discussion

Tubular obstruction is a prominent abnormality following 60 min of unilateral complete renal artery occlusion in the rat. This conclusion has been based on the findings of distended tubular lumina with little or no spontaneous movement of tubular fluid and the presence of markedly elevated PITP [3, 4, 5]. Furthermore, these observations have been correlated with morphologic evidence of tubular epithelial cell injury and necrosis [20, 21] and the demonstration of an obstructive lesion in straight segments of proximal tubules [7].

The presence of tubular obstruction is not an isolated abnormality, in that total renal vascular resistance is increased as reflected by a decrease in renal blood flow. Nonetheless, collection of tubular fluid proximal to the site of obstruction has allowed the measured single nephron filtration rate to return to approximately 70% of normal [3]. This has been explained on the basis of the observation that in the early postischemic period the resistance changes are so distributed across the preglomerular and postglomerular vasculature that both the SFP and GCP are normal or slightly elevated [4, 5]. This situation changes over time. Over a period of 24 hours there is a further increase in preglomerular vascular resistance, which results in a substantial fall in SFP and GCP.

In addition to the presence of tubular obstruction and preglomerular vasoconstriction, tubular epithelial damage permits the passive backflow of filtrate. Tanner, Sloan, and Sophasan [3] found that shortly after the period of ischemia, ipsilateral inulin recovery following microinjections into proximal convolutions was only 36.4%, whereas 27.3% was recovered from the contralateral kidney. Similar findings were obtained by Donohoe et al [7], who also found that some of the injured tubular epithelial cells had become abnormally permeable to larger molecular weight proteins. They observed that when horseradish peroxidase was microinjected into proximal tubular lumina or injected intravenously, it penetrated the cytoplasm of these cells in an abnormal fashion and passed out into the interstitium. Olbricht et al [8] microperfused inulin through pipettes placed in proximal tubules. Inulin recoveries from the distal tubules and from the final urine were significantly less than were those from normal kidneys. Arendshorst, Finn, and Gottschalk [4] found that 24 hours after the ischemic period the loss of integrity of the tubular epithelium had become great enough that small volumes of dye solutions microinjected into proximal convolutions could be seen passing radially from the lumen into the peritubular circulation.

The simultaneous presence of these abnormalities has made it difficult to assess the relative importance of each in the maintenance of the renal failure and to predict the sequence of events leading to recovery. Indeed, tubular obstruction, preglomerular vasoconstriction, and backflow of filtrate can be demonstrated for as long as 1 week following ischemia [6, 19]. Our observations of renal function up to 8 weeks following ischemia indicate that recovery from postischemic acute renal failure in the rat can be separated into two components. The initial increase in C_{In} occurs out of proportion to the change in RBF and is temporally related to the return of anatomical and functional integrity of the

tubular epithelium. Later increases in C_{In} are more gradual and tend to parallel the progressive elevation of RBF and SFP.

By the second week following occlusion, the C_{In} had increased more than tenfold, despite the fact that RBF, SFP, and GCP_e remained depressed. There was, however, marked improvement in the histologic appearance of the tubular epithelial cells and a considerable reduction in the extent of intratubular casts. Moreover, our microinjection data indicate that because the ipsilateral urinary recovery of ^{3}H-inulin at 14 days was complete and not different from control values, restoration of the anatomical integrity of the tubular epithelium for substances with a molecular weight similar to that of inulin had occurred. In addition, we found a slight but significant reduction of the ipsilateral recovery of mannitol, suggesting that, at least in some tubules, recovery of epithelial anatomical integrity to small molecular weight substances was incomplete. These data are consistent with those of Eisenbach and Steinhausen [6], who also found evidence of progressive recovery of the integrity of the tubular epithelium. They found that when inulin was microinjected into the postischemic kidney at days 3 and 4, nearly 100% of the inulin which subsequently appeared in the urine was excreted by the contralateral kidney. During days 5 to 7, the proportion of inulin recovered from the contralateral side was greater than 50% in one half of the animals, but by days 10 to 13, none of the inulin was recovered from the contralateral kidney.

In our studies, as the structural integrity of the tubular epithelium returned, there was progressive improvement in tubular function, as indicated by changes in the pattern of sodium and water excretion. The urinary sodium concentration fell considerably between the first and second weeks following ischemia, and by the fourth and eighth weeks was not different from the urinary sodium concentration of the contralateral nonischemic kidney. Similar patterns were observed for both the fractional excretion of sodium and the fractional excretion of water. Of interest is the comparison of the absolute values of urine flow rates and sodium excretion from the postischemic kidney. As a result of the residual defect in sodium and water reabsorption, the relief of tubular obstruction, and the abrupt increase in C_{In} that were found at 2 weeks, both the urine volume and sodium excretion were greater at this interval than they were at any other time.

The gradual repair of tubular epithelium and decrease in the degree of intratubular obstruction explains in part the various patterns of intratubular hydrostatic pressure. Previous studies [4, 5] have indicated that in the immediate postischemic period, the maintenance of a normal or slightly elevated SFP in the presence of tubular obstruction results in a marked increase in PITP. Over a period of 24 hours, the SFP falls, and together with necrosis of the tubular epithelium, the PITP is reduced. Our studies demonstrate that during the first week as the tubular epithelium is repaired, there is a tendency for the PITP to rise despite the continued reduction of SFP. This rise in PITP is followed by a reduction in the degree of obstruction at 2 weeks and a significant fall in PITP.

The elevation of the PITP at the end of the first week is not by itself sufficient to account for the decrease in intratubular obstruction. Tanner and Steinhausen [22] have found that during the first several days after temporary renal artery occlusion intratubular pressures of 150 to 100 mm Hg are necessary to dislodge the intratubular obstruction. Despite the heterogenity of the PITP and SFP found in our kidneys, values in this range were never observed. Consequently, consolidation of the necrotic debris must have occurred in such a fashion that intratubular resistance decreased. Coupled with a rise in the PITP due to repair of the tubular epithelium, tubular obstruction was relieved.

This sequence of events is responsible for the initial component of recovery. Other factors, perhaps of a more complex nature, contribute to the continued improvement in C_{In} and determine the extent to which this occurs. Between the second and eighth weeks following occlusion, the rate of rise of the C_{In} was similar to that of RBF. In this regard, it is notable that the rise in SFP and GCP_e was not apparent until the latter phases of recovery. Several studies have indicated that a relationship exists between either obstruction to tubular fluid flow or damage to the tubular epithelium and the development of preglomerular vasoconstriction [12, 23–27]. For example, it has been demonstrated that prolonged obstruction to tubular fluid flow induced either by unilateral ureteral ligation [13, 23] or blockage of individual nephrons [13, 24] is associated with a fall in SFP. Furthermore, it has been postulated that the return of renal vascular resistance to normal levels will occur following relief of intratubular obstruction and reestablishment of tubular flow [1]. Although such a pattern was observed in our studies, they do not demonstrate a causal relationship.

It should be pointed out that just as the factors responsible for the initiation and maintenance of

acute renal failure may depend upon the method by which the renal damage is produced, so also may the pattern of recovery differ from model to model. In this regard, Oken, DiBona, and McDonald [28], in studies of glycerol-induced acute renal failure in rats, were unable to find evidence that the gradual return of renal function towards normal reflected a slow release of tubular obstruction or repair of disrupted tubular epithelium. In their studies, recovery appeared to be directly attributable to the return of an adequate effective glomerular filtration pressure.

Further complicating our understanding of the factors responsible for continued recovery is the finding, similar to that of others [29], that temporary renal ischemia results in an eventual loss of renal mass. The weights of the postischemic kidneys fell progressively during the period of observation and by 8 weeks were only two thirds the weight of the contralateral kidney. Preliminary observations [30] have indicated, however, that a loss in renal mass is not an inevitable consequence of temporary renal ischemia and that enhanced structural and functional recovery may occur following removal of the contralateral normal kidney.

Although the precise mechanisms regulating renal size and function during later stages of recovery are not clear, our results do indicate that the onset of recovery from postischemic renal failure in the rat is associated most closely with the repair of the tubular epithelium and the relief of intratubular obstruction. Whereas restoration of RBF to normal rates is not required for the initiation of recovery, the resolution of the abnormalities in renal vascular resistance is a necessary component of the later stages of recovery.

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